

WHAT IS CLAIMED IS:

- 1 1. A method for purifying a polypeptide from a composition comprising the
2 polypeptide and contaminants, which method comprises the sequential steps of:
3 (a) loading the composition onto an ion exchange resin with an equilibration
4 buffer having a first salt concentration;
5 (b) washing the ion exchange resin with a wash buffer until a predetermined
6 protein concentration is measured in the flowthrough, wherein the salt concentration of the
7 wash buffer increases from an initial, second salt concentration that is greater than the salt
8 concentration of the equilibration buffer, to a final, third salt concentration;
9 (c) passing a fixed volume of wash buffer at the final, third salt concentration
10 over the cation exchange resin; and
11 (d) eluting the polypeptide from the ion exchange resin with elution buffer that
12 has a salt concentration that is greater than the final salt concentration of the wash buffer.
- 1 2. The method of claim 1 wherein the ion exchange resin is an anion exchange
2 resin.
- 1 3. The method of claim 1 wherein the ion exchange resin is a cation exchange
2 resin.
- 1 4. The method of claim 3 wherein the cation exchange resin comprises
2 sulphopropyl immobilized on agarose.
- 1 5. The method of claim 1 wherein the elution buffer has a higher conductivity
2 than the equilibration buffer.
- 1 6. The method of claim 1 wherein the elution buffer comprises about 145 mM
2 Na/HOAc and the equilibration buffer comprises about 70 mM Na/HOAc.
- 1 7. The method of claim 1 wherein the elution buffer comprises about 100 mM
2 NaCl and the equilibration buffer comprises about 45 mM NaCl.

- 1 8. The method of claim 1 wherein the wash buffer comprises a mixture of
2 equilibration buffer and elution buffer.
- 1 9. The method of claim 8 wherein the increase in the salt concentration of the
2 wash buffer during step (b) is achieved by increasing the proportion of elution buffer in the
3 wash buffer.
- 1 10. The method of claim 9 wherein the proportion of elution buffer in the wash
2 buffer increases at a constant rate.
- 1 11. The method of claim 10 wherein the increase in the proportion of elution
2 buffer causes the salt concentration of the wash buffer to increase at a constant rate of from
3 about 1 mM to about 3 mM per column volume of wash buffer.
- 1 12. The method of claim 9 wherein the percentage of elution buffer in the wash
2 buffer increases at two or more different rates during the course of washing in step (b).
- 1 13. The method of claim 12 wherein the percentage of elution buffer in the wash
2 buffer increases at a first rate for a first segment of the washing, at a second rate for a second
3 segment of the washing and at a third rate for a third segment of the washing.
- 1 14. The method of claim 1 wherein the polypeptide is an antibody.
- 1 15. The method of claim 14 wherein the antibody binds HER2.
- 1 16. The method of claim 14 wherein the contaminant is a deamidated variant of
2 the antibody.
- 1 17. The method of claim 14 wherein the amount of antibody in the composition
2 loaded onto the ion exchange resin is from about 15 mg to about 45 mg per mL of cation
3 exchange resin.
- 1 18. The method of claim 1 wherein the predetermined protein concentration in
2 step (b) corresponds to an OD of 0.6 measured at 280 nm.

- 1 19. The method of claim 1 wherein from about 0.4 to about 1 column volumes of
2 wash buffer are passed over the ion exchange resin in step (c).
- 1 20. The method of claim 1 wherein the pH of the equilibration buffer, wash buffer
2 and elution buffer is approximately the same.
- 1 21. The method of claim 23 wherein the pH of the equilibration buffer, wash
2 buffer and elution buffer is approximately 5.5.
- 1 22. The method of claim 1 further comprising subjecting the composition
2 comprising the polypeptide to one or more further purification steps either before, during, or
3 after steps (a) through (d) so as to obtain a homogeneous preparation of the polypeptide.
- 1 23. The method of claim 22 further comprising preparing a pharmaceutical
2 composition by combining the homogeneous preparation of the polypeptide with a
3 pharmaceutically acceptable carrier.
- 1 24. The method of claim 22 further comprising conjugating the purified
2 polypeptide with a heterologous molecule.
- 1 25. The method of claim 24 wherein the heterologous molecule is polyethylene
2 glycol, a label or a cytotoxic agent.
- 1 26. A polypeptide which has been purified according to the method of claim 1.
- 1 27. A method for purifying an antibody from a composition comprising the
2 polypeptide and a contaminant, which method comprises the following steps performed
3 sequentially:
4 (a) binding the antibody to a cation exchange material with an equilibration
5 buffer at a first conductivity;
6 (b) washing the cation exchange material with a wash buffer, wherein the
7 conductivity of the wash buffer increases from a second conductivity that is higher than the
8 first conductivity to a third conductivity during the washing;

9 (c) passing a fixed volume of wash buffer at the third conductivity over the
10 cation exchange material; and

11 (d) eluting the antibody from the cation exchange material with an elution
12 buffer at a fourth conductivity that is higher than the third conductivity.

1 28. The method of claim 27 wherein the cation exchange resin comprises
2 sulphopropyl immobilized on agarose.

1 29. The method of claim 27 wherein the conductivity of the wash buffer increases
2 at a constant rate from the second conductivity to the third conductivity.

1 30. The method of claim 27 wherein the conductivity of the wash buffer increases
2 at two or more different rates from the second conductivity to the third conductivity.

1 31. The method of claim 30 wherein the conductivity of the wash buffer increases
2 at a first rate for a first segment of the washing, at a second rate for a second segment of the
3 washing and at a third rate for a third segment of the washing.

1 32. The method of claim 31 wherein the wash buffer comprises a mixture of
2 equilibration buffer and elution buffer.

1 33. The method of claim 32 wherein the conductivity of the wash buffer is
2 increased by increasing the proportion of elution buffer in the wash buffer.

1 34. The method of claim 33 wherein the proportion of elution buffer in the wash
2 buffer increases at a constant rate of about 6% during the first segment, at a constant rate of
3 about 3.5% during the second segment and at a constant rate of about 2% during the third
4 segment.

1 35. The method of claim 33 wherein the proportion of elution buffer in the wash
2 buffer increases from about 26% to about 54% during the first segment, from about 54% to
3 about 61% during the second segment and from about 61% to about 74% during the second
4 segment.

36. The method of claim 31 wherein the cation exchange material is washed with about 5 column volumes of wash buffer in the first segment, about 2 column volumes of wash buffer in the second segment and about 6 column volumes of wash buffer in the third segment.

37. The method of claim 27 wherein the conductivity of the wash buffer is increased by increasing the percentage of elution buffer in the wash buffer.

38. The method of claim 27 wherein the conductivity of the wash buffer is increased by increasing the salt concentration therein.

39. The method of claim 27 wherein the fixed volume of wash buffer passed over the cation exchange material in step (c) is between about 0.4 column volumes and about 1.0 column volumes.

40. The method of claim 27 further comprising washing the ion exchange material with a regeneration buffer after step (d).

41. A method for purifying an antibody from a composition comprising the antibody and a contaminant, which method comprises the following steps performed sequentially:

(a) loading the composition onto a cation exchange material;

(b) washing the cation exchange material with a wash buffer with a conductivity that increases at a first rate from a first conductivity to a second conductivity, at a second rate from the second conductivity to a third conductivity and at a third rate from the third conductivity to a fourth conductivity; and

(c) eluting the antibody from the ion exchange material,

wherein the amount of antibody in the composition loaded onto the cation exchange material is from about 15 mg to about 45 mg of the antibody per ml of cation exchange material.

42. A method for purifying a polypeptide from a composition comprising the polypeptide and a contaminant, which method comprises the following steps performed sequentially:

(a) loading the composition onto an ion exchange material;

5 (b) washing the cation exchange material with wash buffer using a multi-slope
6 gradient until a predetermined protein concentration is measured in the flowthrough; and
7 (c) eluting the polypeptide from the ion exchange material.

1 43. The method of claim 42 wherein the multi-slope gradient comprises two or
2 more segments.

1 44. The method of claim 43 wherein each segment of the multi-slope gradient has
2 a shallower slope.

1 45. The method of claim 42, additionally comprising the step between steps (b)
2 and (c) of washing the column with from 0.4 to 1 column volumes of wash buffer.

1 46. The method of claim 45 wherein the wash buffer has the composition of the
2 wash buffer at the end of step (b).